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Of mice and men

Fuzzy tandem repeats and divergent p53 transcriptional repertoires

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The clinical importance of tumor suppressor p53 makes it one of the most studied transcription factors. A comparison of mammalian p53 transcriptional repertoires may help identify fundamental principles in genome evolution and better understand cancer processes. Here we summarize mechanisms underlying the divergence of mammalian p53 transcriptional repertoires, with an emphasis on the rapid evolution of fuzzy tandem repeats containing p53 response elements.

Keywords: p53, transcriptional repertoires, response element, genome evolution, fuzzy tandem repeat, retinoblastoma

Abbreviations: FTR, fuzzy tandem repeat; RE, response element; BS, binding site; LTR, long terminal repeat; ChIP, chromatin immunoprecipitation; ChIP-seq, ChIP followed by sequencing; LINE, long interspersed nuclear element; SINE, short interspersed nuclear element; ERV, endogenous retrovirus; *PIG3*, P53-induced gene 3; *Rbl2*, retinoblastoma like 2; MEF, mouse embryonic fibroblast; TSS, transcription start site; BLAST, basic local alignment search tool; miRNA, microRNA; S-p130, shorter isoform of p130

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Introduction

Phenotype evolution was long thought to rely mainly on the evolution of protein-coding sequences. In recent years, however, global genome sequencing of various species highlighted the high level of conservation of coding sequences between species, and the idea emerged that regulatory regions may play a critical role in evolution. It is now well accepted that changes in gene expression – mainly due to modification in cis-regulatory elements – underlie many of the phenotypic differences between species.[1,2] Different unstable elements can be present in regulatory regions and modulate the transcriptional efficiency of enhancers and promoters. This is also true for the p53 pathway, which plays a crucial role in development, genome integrity and tumor suppression.

The p53 tumor suppressor pathway is deregulated—through mutation of the *TP53* gene or modifications of its regulators or effectors—in most if not all human tumors. p53 mainly acts as a transcription factor that binds specific response elements (REs) located in the promoters or intronic sequences of its target genes. The consensus sequence for a p53 RE was initially defined as 2 copies of the half-site motif RRRCWWGYYY (where R = G/A, W = A/T, Y = C/T) separated by 0–13 bp.[3] Recent whole-genome ChIP-seq approaches further refined this consensus and suggested more precise positional frequency matrices.[4,5] The conservation between mice and humans of functional p53 RE(s) within regulatory sequences of a candidate gene has often been used to strengthen the notion that the gene of interest was a *bona fide* p53 transcriptional target, and that its regulation by p53 was biologically relevant (See ref. 6 for an early example, and ref. 7 for a more recent one). While this approach led to the discovery of many important p53 targets, it also probably led to an under-appreciation of the differences between human and murine p53 transcriptional repertoires. We discuss below the different mechanisms that may account for the divergence of mammalian p53 transcriptional repertoires.

Repeated Sequences and Divergent Transcriptional Repertoires

More than half of the human genome is composed of repeated sequences known as long and short interspersed nuclear elements (LINEs and SINEs) that result from replicative copy and paste events of retrotransposons. Although most retrotransposons are now fixed in human cells, a few L1 LINEs and SINEs (mainly primate-specific Alu sequences) are still

mobile.[8] Several arguments suggest that retrotransposons contributed to shape the human p53 transcriptional repertoire. Wang et al. showed that 1,509 of ≈319,000 LTR class I endogenous retrovirus (ERV) in the human genome have a near-perfect p53 RE. These ERV are primate specific and can induce the transcription of nearby genes.[9] p53 REs were also found in L1 elements,[10] and 24 out of the 157 demonstrated p53 REs are located within Alu repeats.[11] Similarly, a fish-specific transposable element was recently found to have shaped the p53 transcriptional repertoire of the Zebrafish.[12]

In addition to transposable elements, a microsatellite repeat was reported to cause differential p53 regulation. Human p53 was shown to mediate the transactivation of *PIG3*, the p53-induced gene 3, through binding to the microsatellite $(TGYCC)_n$. It was proposed that the low resemblance between this microsatellite and a canonical p53 RE was compensated by the large number of p53 binding sites resulting from the repeats.[13] Importantly, microsatellites are extremely unstable in length, with mutation rates 10,000 to 100,000 times higher than average mutation rates in the genome.[14] The *PIG3* promoter acquired p53 responsiveness due to the instability of the TGYCC microsatellite: *PIG3* was shown to be a p53 target gene in Hominidae (human and apes) but not in monkeys because the *PIG3* promoter contains 14–19 copies of the TGYCC repeat in the former group, but only 5–6 copies in the latter group.[15] In humans the polymorphism of this microsatellite might also have a clinical significance, as suggested by the higher grade in invasive bladder cancers for patients with 14 or less TGYCC repeats.[16]

Our team recently described a new class of repeated sequences that display low inter-species conservation and are involved in shaping p53 transcriptional repertoires. We identified three p53 target genes induced by the binding of p53 to clusters of canonical p53 REs within imperfect repeats called "Fuzzy Tandem Repeats" (FTRs). Although FTRs are likely more stable than perfect tandem repeats,[17] the sequences of the FTRs diverged among mammalian species, and the 3 genes were identified as p53 transcriptional targets in murine/rodent cells but not in human cells.[18] As summarized below, differences between humans and mice in the mutations required to initiate retinoblastoma were the starting point of these findings.

Rbl2/p130 is a p53 Target Gene in Mouse Cells

The genetic events leading to retinoblastomas differ between humans and mice. While humans carrying a germline *RB1* mutation are predisposed to develop retinoblastomas, the deletion of one copy of *Rb1* in mice does not lead to retinoblastomas, but rather to thyroid and pituitary tumors.[19] The development of retinoblastomas in mice requires a concomitant loss of Rb and one of the Rb-like proteins (p107 or p130) in the retina.[20-23] It was suggested that *Rb1* loss in the mouse retina does not lead to retinoblastoma due to a compensatory upregulation of p107 and a partially redundant expression of Rb and p130.[24-26] We were intrigued by the fact that p107-deficient mice with an *Rb1* deletion in the retina develop non invasive retinoblastomas with low penetrance, whereas similar mice with an additional retina-specific loss of p53, or with combined decrease in p107/p130 levels, developed aggressive and invasive bilateral retinoblastomas.[26-28] These data led us to suspect a functional link between p53 and p130 in the mouse, which could be important to prevent retinoblastoma formation in this species.

Consistent with this, we found that *Rbl2*/p130 is induced in a p53-dependent manner in mouse embryonic fibroblasts (MEFs) and various mouse tissues, but not in human fibroblasts.[18] We used a positional frequency matrix to search for putative p53 REs at the *Rbl2* locus *in silico* and were surprised to find, about 1.8 kb downstream of the p130 transcription start site, a cluster of 4 putative p53 REs, two of which perfectly matched the canonical sequence for a RE (**Fig. 1A**). We next used ChIP experiments and reporter luciferase assays to show that these clustered p53 REs are crucial for the transactivation of p130 by p53.[18] Together, these data demonstrated that *Rbl2*/p130 is a *bona fide* p53 target gene in mouse cells.

Fuzzy Tandem Repeats Containing p53 REs are Poorly Conserved among Mammals

Importantly, two of the clustered p53 REs in mouse *Rbl2*/p130 had identical sequences, suggesting that a duplication participated in the creation of this cluster. Indeed *mreps*, a software designed to identify repeats, detected fuzzy tandem repeats (i.e., tandem repeats with a few mismatches) encompassing the entire cluster of p53REs (**Fig. 1B**). Tandem repeats are known for their high instability and low conservation among individuals and species. However, the purity of the repeats is highly correlated with the mutation rate, and even a few mismatches can dramatically enhance the stability of the repeat.[17] Therefore, the FTRs in the mouse *Rbl2* gene were predicted to evolve more rapidly than non-repeated DNA sequences, but were likely more stable than perfect tandem repeats.

Consistent with this, when we analyzed the cluster of p53 REs in 17 mouse strains,[29] we found very few polymorphisms between the strains, all of which were unlikely to affect the p53-dependent regulation of p130 (data not shown). On the opposite, when we searched for p53 REs 1.5–2 kb downstream of the transcription start site in the *Rbl2* gene from other mammalian species, we found a single p53 RE in the rat gene—indicating a partial conservation among rodents, but no p53 RE in the other species (**Fig. 1C**).[18] In humans, the region homologous to the murine cluster of p53 REs contained degenerated p53 binding half-sites, consistent with the fact that p53 does not transactivate p130 in human cells.[18]

We hypothesized that other p53 target genes could be regulated by p53 via clusters of p53 REs and performed BLAST searches over the entire mouse genome with the *Rbl2* p53 RE cluster, or with theoretical clusters of p53 REs. This led to identify 2 additional p53 target genes: *Ncoa1* and *Klhl26*.[18] As for *Rbl2*, the clustered p53 REs at the *Ncoa1* and *Klhl26* loci are contained within FTRs whose sequences are poorly conserved among mammalian genomes (**Fig. 1D**). The clustered p53 REs at the *Klhl26* locus were

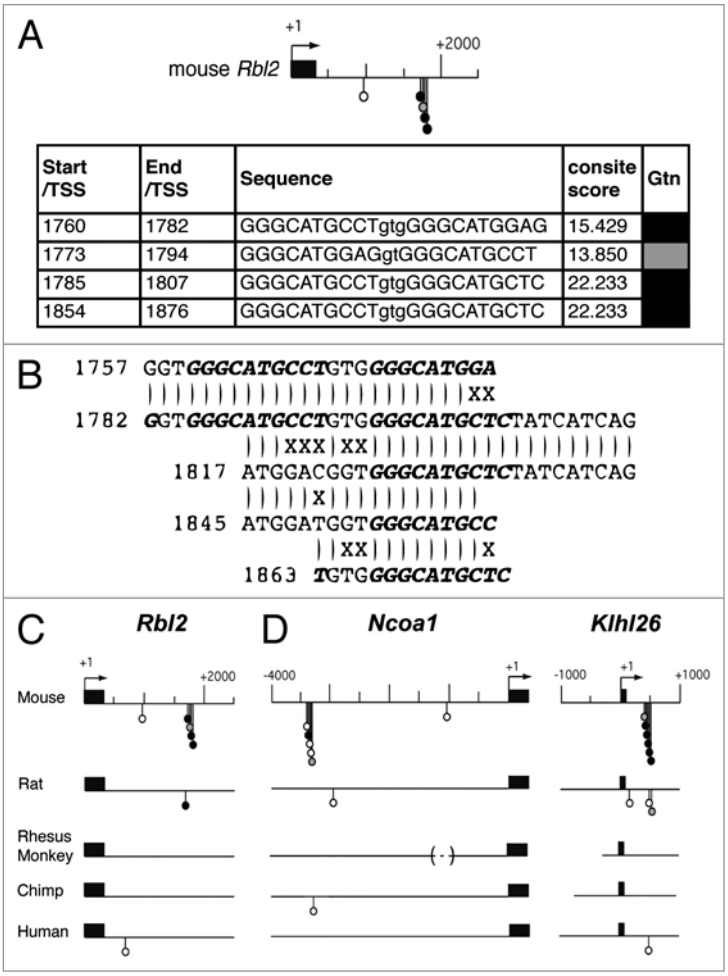
partially conserved in the rat genome, but not in primate genomes. Accordingly, p53 transactivated *Klhl26* in mouse and rat cells, but not in human cells. The FTRs containing clustered p53 REs at the *Ncoa1* locus appeared to evolve even more rapidly, and this gene was transactivated by p53 only in mouse cells. Together, these results revealed that the rapid evolution of fuzzy tandem repeats containing p53 REs define a subset of species-specific p53 target genes.[18]

Further Analysis of the p53-Mediated Regulation of *Rbl2*

We and others have found that p130 is not transactivated by p53 in human cells.[18,30] We were thus initially surprised by the data from a recent genome-wide ChIP-seq study which identified *Rbl2* as a novel p53-target gene in human MCF7 cells.[31] However a closer examination of the ChIP-seq data provided an explanation for this apparent contradiction, as p53 was reported to bind within the intron 4 of human *Rbl2*.[31] Importantly, Gao et al. previously found that in Rb-deficient Saos2 cells p53 binds a RE within the intron 4 of human *Rbl2* to induce a N-terminally truncated isoform of p130 called S-p130 (S standing for Short).[32]

These results led us to further analyze the regulation of *Rbl2* by p53 in mouse cells. We identified a putative p53 RE in the intron 4 of mouse *Rbl2* (**Fig. 2A**), and found that p53 activation in MEFs induces a p130 mRNA isoform which, like the human mRNA encoding S-p130, retains part of the *Rbl2* intron 4 (**Fig. 2B**). The binding of murine p53 on the p53 RE within intron 4 was then confirmed by both ChIP and luciferase reporter assays (**Fig. 2C and D**).

Together, these results suggest that in mouse cells p53 transactivates *Rbl2* to produce two isoforms: p130 via clustered p53 REs within FTRs in the intron 1, and S-p130 via a single p53 RE in the intron 4. In human cells, p53 activation would only induce S-p130, via the p53 RE in the intron 4. Further analyses will be needed to determine the functions of the S-p130 isoform, in both human and mouse cells.



Start /TSS	End /TSS	Sequence	consite score	Gtn
1760	1782	GGGCATGCCTgtgGGGCATGGAG	15.429	
1773	1794	GGGCATGGAGgtGGGCATGCCT	13.850	
1785	1807	GGGCATGCCTgtgGGGCATGCTC	22.233	
1854	1876	GGGCATGCCTgtgGGGCATGCTC	22.233	

Figure 1. Murine p53 transactivates *Rbl2*/p130, *Ncoa1* and *Klhl26 via* clustered p53 REs within rapidly evolving fuzzy tandem repeats. (**A**) A search for putative p53 REs at the mouse *Rbl2* locus was performed *in silico* by using *Consite* (http://asp.ii.uib.no:8090/cgi-bin/CONSITE/consite) and a positional frequency matrix. The 2.5 kb downstream of the p130 mRNA transcription start site (TSS) are shown, with putative p53 REs plotted along the *Rbl2* locus as lollipops and greytones according to *Consite* scores (black for scores > 15.3, gray for scores between 12.9 and 15.3, and white for scores between 10.5 and 12.9). Black box: exon 1. The table below the map details the sequences and positions of the clustered candidate p53 REs (Gtn: greytone according to *Consite* score). For detailed methods, see ref. 18. (**B**) Integrated results of the *mreps* analysis (http://bioinfo.lifl.fr/mreps/mreps.php. resolution = 5; error rate < 0.2) of the clustered p53 REs within *Rbl2* intron 1. The p53 half-sites are represented in bold italics. Numbers are relative to the TSS. (**C**) Putative p53 REs were searched for, by using *Consite* as in A, at the *Rbl2* locus of several mammalian species (only rodents and primates are shown here – for additional species and detailed sequences, see ref. 18). (**D**) Putative p53 REs were searched for, as in A, at the *Ncoa1* and *Klhl26* loci of several mammalian species (again, see ref. 18 for additional species and detailed sequences).

Conclusions and Perspectives

Evidence that transposable elements played a major role in shaping differences between mammalian p53 transcriptional repertoires has accumulated in recent years. We recently found another mechanism that may generate such differences: the rapid evolution of fuzzy tandem repeats containing p53 response elements. So far, we identified only three genes belonging to this new class of species-specific target genes: *Rbl2*, *Ncoa1* and *Klhl26*. Importantly, however, current software programs were not designed to search for imperfectly repeated sequences over entire genomes, and we limited our functional validation of candidate p53 targets to genes that are expressed in MEFs. We thus expect that more examples of species-specific p53 target genes regulated via FTRs will be found in the near future.

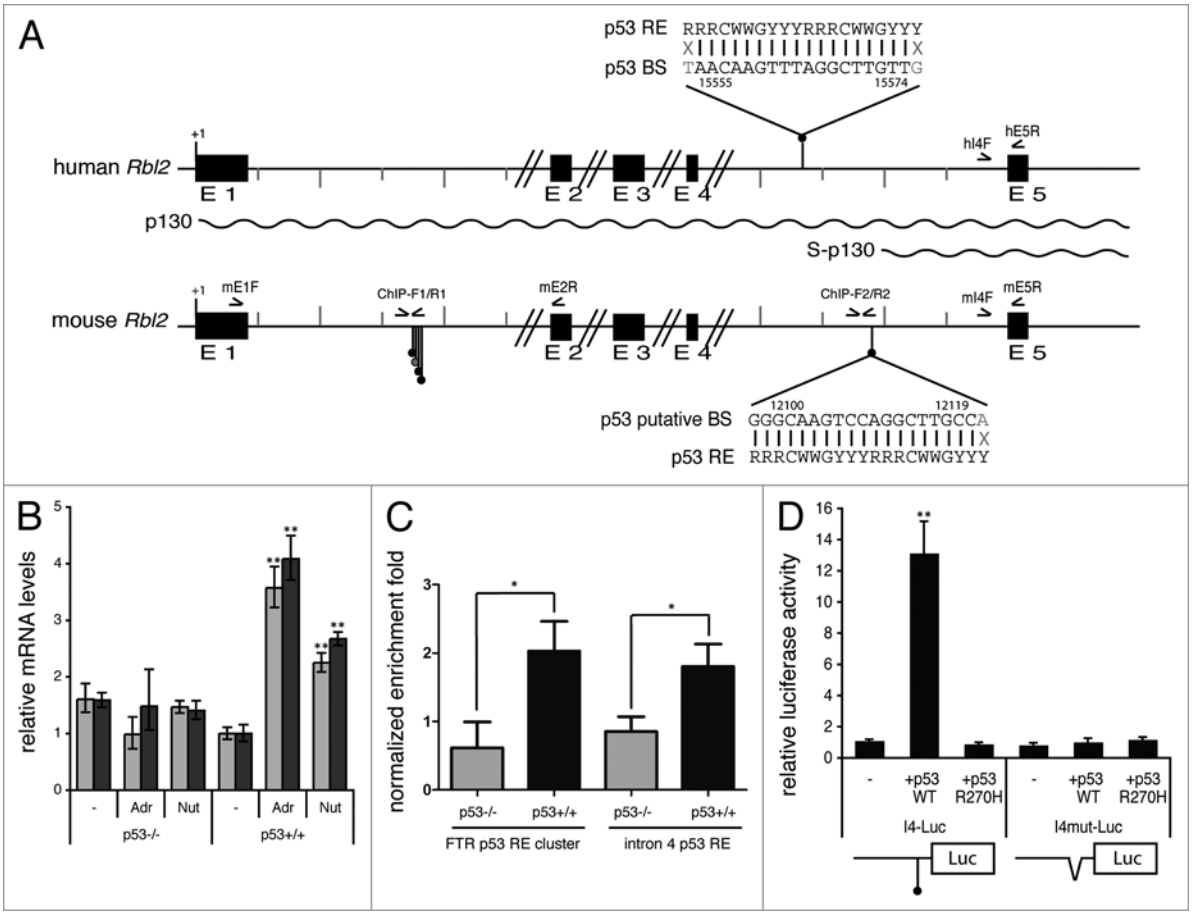


Figure 2. Murine p53 transactivates *Rbl2 via* a p53 RE in intron 4 to produce an alternative transcript. (**A**) Partial genomic structure of human and mouse *Rbl2*. p53 REs (Consite scores > 12.9) are represented as lollipops as in **Figure 1**. The consensus sequence for a p53 RE and for p53 binding sites (p53 BS) within introns 4 are shown. Numbers are relative to the TSS of the mRNA for the full-length p130 isoform. Arrows represent primers used here and in ref. 32. (**B**) WT and p53$^{-/-}$ MEFs were left untreated or treated for 24 h with 0.5 μg/ml Adriamycin (Adr) or 20 μM Nutlin 3a (Nut) prior to RNA extraction and real-time PCR quantification. Relative mRNA levels were quantified by real-time PCR. Light gray: p130 mRNAs were quantified with primers mE1F and mE2R (represented in (**A**), normalized to control mRNAs, then a value of 1 was assigned to mRNA amounts in unstressed WT cells. Dark gray: S-p130 mRNAs were quantified with primers mI4F (CTT GGT ATA ATG GTG TCT GGT GTC) and mE5R (GGG TTC ACA AGT TCT TTA CGG), normalized to control mRNAs, then a value of 1 was assigned to mRNA amounts in unstressed WT cells. Notice that, assuming similar PCR efficiency for the primer sets quantifying each p130 isoform, mRNAs encoding the S-p130 isoform are about 18 times less abundant than mRNAs encoding the full-length p130 isoform. (**C**) ChIP assay was performed on Adriamycin-treated MEFs, with an antibody against p53 (FL393, Santa-Cruz), or rabbit IgG as a control. Immunoprecipitates were quantified by RT-PCR and normalized to background immunoprecipitation by rabbit IgG. The normalized enrichment fold is calculated as p130 immunoprecipitates - primers ChIP-F1/R1 (represented in (**A**), sequences in ref. 18) or ChIP-F2/R2 (TGT CAC ATG AAG GGC TTT TTG A and CAA AAC ATT TCC GGT GTC ATG A) -against an irrelevant region. (**D**) The 3' half of intron 4 of mouse *Rbl2* was cloned before a luciferase reporter gene preceded by a minimum promoter (I4-Luc). The same vector was constructed with the deletion of the indicated p53-RE (I4mut-Luc). These plasmids were transfected in p53$^{-/-}$ MEFs alone or with an expression vector for WT p53 (+p53WT) or mutant p53 (+p53R270H) to measure firefly luciferase activity. Results were normalized to control renilla luciferase. In all figures **p ≤ 0.01 and *p ≤ 0.05 by Student's t-test. For detailed methods and additional primer sequences, see ref. 18.

It will also be important to determine the impact of FTRs on shaping the target gene repertoires of other transcription factors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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References

1. Brawand D, Soumillon M, Necsulea A, Julien P, Csárdi G, Harrigan P, et al. The evolution of gene expression levels in mammalian organs. Nature 2011; 478:343-8; PMID:22012392; http://dx.doi.org/10.1038/nature10532.
2. Wittkopp PJ, Kalay G. Cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence. Nat Rev Genet 2012; 13:59-69; PMID:22143240.
3. el-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. Definition of a consensus binding site for p53. Nat Genet 1992; 1:45-9; PMID:1301998; http://dx.doi.org/10.1038/ng0492-45.
4. Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. Nat Rev Mol Cell Biol 2008; 9:402-12; PMID:18431400; http://dx.doi.org/10.1038/nrm2395.

5. Smeenk L, van Heeringen SJ, Koeppel M, van Driel MA, Bartels SJ, Akkers RC, et al. Characterization of genome-wide p53-binding sites upon stress response. Nucleic Acids Res 2008; 36:3639-54; PMID:18474530; http://dx.doi.org/10.1093/nar/gkn232.
6. el-Deiry WS, Tokino T, Waldman T, Oliner JD, Velculescu VE, Burrell M, et al. Topological control of p21WAF1/CIP1 expression in normal and neoplastic tissues. Cancer Res 1995; 55:2910-9; PMID:7796420.
7. Hu W, Feng Z, Teresky AK, Levine AJ. p53 regulates maternal reproduction through LIF. Nature 2007; 450:721-4; PMID:18046411; http://dx.doi.org/10.1038/nature05993.
8. Burns KH, Boeke JD. Human transposon tectonics. Cell 2012; 149:740-52; PMID:22579280; http://dx.doi.org/10.1016/j.cell.2012.04.019.
9. Wang T, Zeng J, Lowe CB, Sellers RG, Salama SR, Yang M, et al. Species-specific endogenous retroviruses shape the transcriptional network of the human tumor suppressor protein p53. Proc Natl Acad Sci U S A 2007; 104:18613-8; PMID:18003932; http://dx.doi.org/10.1073/pnas.0703637104.
10. Harris CR, Dewan A, Zupnick A, Normart R, Gabriel A, Prives C, et al. p53 responsive elements in human retrotransposons. Oncogene 2009; 28:3857-65; PMID:19718052; http://dx.doi.org/10.1038/onc.2009.246.
11. Cui F, Sirotin MV, Zhurkin VB. Impact of Alu repeats on the evolution of human p53 binding sites. Biol Direct 2011; 6:2; PMID:21208455; http://dx.doi.org/10.1186/1745-6150-6-2.
12. Micale L, Loviglio MN, Manzoni M, Fusco C, Augello B, Migliavacca E, et al. A fish-specific transposable element shapes the repertoire of p53 target genes in zebrafish. PLoS One 2012; 7:e46642; PMID:23118857; http://dx.doi.org/10.1371/journal.pone.0046642.
13. Contente A, Dittmer A, Koch MC, Roth J, Dobbelstein M. A polymorphic microsatellite that mediates induction of PIG3 by p53. Nat Genet 2002; 30:315-20; PMID:11919562; http://dx.doi.org/10.1038/ng836.
14. Vinces MD, Legendre M, Caldara M, Hagihara M, Verstrepen KJ. Unstable tandem repeats in promoters confer transcriptional evolvability. Science 2009; 324:1213-6; PMID:19478187; http://dx.doi.org/10.1126/science.1170097.
15. Contente A, Zischler H, Einspanier A, Dobbelstein M. A promoter that acquired p53 responsiveness during primate evolution. Cancer Res 2003; 63:1756-8; PMID:12702557.
16. Ito M, Nishiyama H, Watanabe J, Kawanishi H, Takahashi T, Kamoto T, et al. Association of the PIG3 promoter polymorphism with invasive bladder cancer in a Japanese population. Jpn J Clin Oncol 2006; 36:116-20; PMID:16418181; http://dx.doi.org/10.1093/jjco/hyi225.
17. Legendre M, Pochet N, Pak T, Verstrepen KJ. Sequence-based estimation of minisatellite and microsatellite repeat variability. Genome Res 2007; 17:1787-96; PMID:17978285; http://dx.doi.org/10.1101/gr.6554007.
18. Simeonova I, Lejour V, Bardot B, Bouarich-Bourimi R, Morin A, Fang M, et al. Fuzzy tandem repeats containing p53 response elements may define species-specific p53 target genes. PLoS Genet 2012; 8:e1002731; PMID:22761580; http://dx.doi.org/10.1371/journal.pgen.1002731.
19. Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. Effects of an Rb mutation in the mouse. Nature 1992; 359:295-300; PMID:1406933; http://dx.doi.org/10.1038/359295a0.
20. Robanus-Maandag E, Dekker M, van der Valk M, Carrozza ML, Jeanny JC, Dannenberg JH, et al. p107 is a suppressor of retinoblastoma development in pRb-deficient mice. Genes Dev 1998; 12:1599-609; PMID:9620848; http://dx.doi.org/10.1101/gad.12.11.1599.
21. Chen D, Livne-bar I, Vanderluit JL, Slack RS, Agochiya M, Bremner R. Cell-specific effects of RB or RB/p107 loss on retinal development implicate an intrinsically death-resistant cell-of-origin in retinoblastoma. Cancer Cell 2004; 5:539-51; PMID:15193257; http://dx.doi.org/10.1016/j.ccr.2004.05.025.
22. MacPherson D, Sage J, Kim T, Ho D, McLaughlin ME, Jacks T. Cell type-specific effects of Rb deletion in the murine retina. Genes Dev 2004; 18:1681-94; PMID:15231717; http://dx.doi.org/10.1101/gad.1203304.
23. Zhang J, Schweers B, Dyer MA. The first knockout mouse model of retinoblastoma. Cell Cycle 2004; 3:952-9; PMID:15190215; http://dx.doi.org/10.4161/cc.3.7.1002.
24. Spencer C, Pajovic S, Devlin H, Dinh QD, Corson TW, Gallie BL. Distinct patterns of expression of the RB gene family in mouse and human retina. Gene Expr Patterns 2005; 5:687-94; PMID:15939381; http://dx.doi.org/10.1016/j.modgep.2005.02.003.
25. Donovan SL, Schweers B, Martins R, Johnson D, Dyer MA. Compensation by tumor suppressor genes during retinal development in mice and humans. BMC Biol 2006; 4:14; PMID:16672052; http://dx.doi.org/10.1186/1741-7007-4-14.
26. Ajioka I, Martins RA, Bayazitov IT, Donovan S, Johnson DA, Frase S, et al. Differentiated horizontal interneurons clonally expand to form metastatic retinoblastoma in mice. Cell 2007; 131:378-90; PMID:17956737; http://dx.doi.org/10.1016/j.cell.2007.09.036.
27. Dyer MA, Rodriguez-Galindo C, Wilson MW. Use of preclinical models to improve treatment of retinoblastoma. PLoS Med 2005; 2:e332; PMID:16231976; http://dx.doi.org/10.1371/journal.pmed.0020332.
28. Wikenheiser-Brokamp KA. Retinoblastoma family proteins: insights gained through genetic manipulation of mice. Cell Mol Life Sci 2006; 63:767-80; PMID:16465443; http://dx.doi.org/10.1007/s00018-005-5487-3.
29. Keane TM, Goodstadt L, Danecek P, White MA, Wong K, Yalcin B, et al. Mouse genomic variation and its effect on phenotypes and gene regulation. Nature 2011; 477:289-94; PMID:21921910; http://dx.doi.org/10.1038/nature10413.
30. Helmbold H, Kömm N, Deppert W, Bohn W. Rb2/p130 is the dominating pocket protein in the p53-p21 DNA damage response pathway leading to senescence. Oncogene 2009; 28:3456-67; PMID:19648966; http://dx.doi.org/10.1038/onc.2009.222.
31. Nikulenkov F, Spinnler C, Li H, Tonelli C, Shi Y, Turunen M, et al. Insights into p53 transcriptional function via genome-wide chromatin occupancy and gene expression analysis. Cell Death Differ 2012; 19:1992-2002; PMID:22790872; http://dx.doi.org/10.1038/cdd.2012.89.
32. Gao CF, Ren S, Wang J, Zhang SL, Jin F, Nakajima T, et al. P130 and its truncated form mediate p53-induced cell cycle arrest in Rb(-/-) Saos2 cells. Oncogene 2002; 21:7569-79; PMID:12386819; http://dx.doi.org/10.1038/sj.onc.1205933.